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ORAL INGESTION OF SYLOID TO MICE AND RATS AND ITS CHRONIC TOXICITY AND CARCINOGENICITY

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ABSTRACT

A long-term bioassay of chronic toxicity was conducted by orally administering the test material of food grade micronized silica, SYLOID (commercially produced by Fuji Davison Chemical Ltd., Japan), in a feed to $B_6C_3F_1$ mice and Fisher rats. The results of this study show that continuous dietary administration of SYLOID, at dose levels of 0, 1.25, 2.5, and 5%, to five-week-old specimens of both sexes for ca 93 consecutive weeks for mice and for ca 103 consecutive weeks for rats, causes no biological or any other meaningful alterations in body weight, food consumption, or physical features. No significant dose-related effects were seen at any dose level upon clinical laboratory examinations. Dietary administration of SYLOID to animals for the duration of a lifespan did not cause gross or microscopic changes in the tissues examined. The occasional presence of some neoplasms did not reveal any consistent, dose-related trends in any group. It may be concluded that proper dietary administration of micronized silica has proven to be generally safe with no long-term toxic effects.

INTRODUCTION

Silica is the most abundant element in the earth's crust, and trace amounts appear in most animal and plant tissues. Despite the use of silica in a wide variety of anti-caking agents for powdered food throughout the world, little has been published on the tox-

icological and carcinogenetic findings based on long-term ingestion. McClendon et al.[1] reported that 10% of silicon fed to rats continuously for 3 months did not have pathological effects on the gastro-intestinal tract, liver, spleen, pancreas, adrenals, lungs, or mesenteric lymph nodes. INBIFO[2] also reported the same results from feeding SYLOID (commercially available product of silica) to mice and rats in low dosage (standard diet with 3.2%) and high dosage (standard diet with 10%) for 6 months. According to Schwarz and Milne[3], silica has growth-promoting effects for animals. Carlisle[4] reported that silica has been demonstrated to be an essential trace element in vertebrae. Although great importance has been attached to the study of certain fibrous silicates, the main involvement of silica in silicosis, there has been relatively little work concerning the effect of silicon on normal metabolism. Evidence that silica dust is harmful to man is based largely upon epidemiological investigations of miner's phthisis and other allied forms of occupational tuberculosis (King and Belt[5]). The purpose of this study is to establish the safety of silicon for use in food as an anti-caking agent for human consumption by characterizing and evaluating the chronic oral, long-term toxicity of SYLOID in rodents. In addition, several carcinogenic studies were carried out as a preliminary test. Furthermore, high oral dosages for a 21-24-month period were used in order to establish high safety ratios. This study began on May 7, 1984, and was completed on May 8, 1986.

Materials and Methods

Essentially the same experimental design was used for both the mouse and the rat.

Test materials: SYLOID 244 (Fuji Davison Chemical. Ltd., Japan) is a fine white silica powder with the chemical composition of $SiO_2 \cdot x\ H_2O$. Lot No. JC-2108 was used throughout this study.

Animals and housing: Three hundred and twenty $B_6C_3F_1$ mice, 160 of each sex, and 320 Fisher rats, 160 of each sex, were purchased from Funabashifarm Animal Co. Ltd., Japan. The mice were 4 weeks old, the rats, 3 weeks. The animals were housed in wire-mesh cages (mice: 5 animals/cage; rats: 2 animals/cage) and prior to initiation of treatment were acclimated to the laboratory environment for 1 week for mice and 2 weeks for rats. At initiation of treatment, the male-mice ranged from 21.0 g to 27.3 g, the female-mice from 16.0 g to 19.9 g; the male-rats ranged from 117 g to 150 g, while the female-rats ranged from 92.0 g to 126 g. Tap water was available ad libitum. Animal quarters were air-conditioned with thermostats set to maintain 23 ± 1 ℃ room temperature continuously and 50 ± 10% humidity; artificial fluorescent lighting was provided daily for a continuous 14-hour period.

Experimental design: Animals were separated according to sex, and by standard randomization 5 mice were put in one cage and 2 rats per cage. Mice and rats were dined into dosage groups of 10 animals each (as shown in Table 1). The test materials, which were prepared weekly were administered orally each day at the prescribed dosage levels.

Table 1. Experimental design of chronic toxicity test with carcinogen observation of SYLOID

Mice

Sex	group	SiO_2 content(ppm)	No. of animals			
			6 monhs	12 months	21 months	total
Male	A	0	10	10	20	40
	B	12,500	10	10	20	40
	C	25,000	10	10	20	40
	D	50,000	10	10	20	40
Female	A	0	10	10	18	38
	B	12,500	10	10	20	40
	C	25,000	10	10	20	40
	D	50,000	10	10	20	40

Rats

Sex	group	SiO_2 content(ppm)	No. of animals			
			6 months	12 months	24 months	total
Male	A	0	10	10	20	40
	B	12,500	10	10	20	40
	C	25,000	10	10	20	40
	D	50,000	10	10	21	41
Female	A	0	10	10	20	40
	B	12,500	10	10	20	40
	C	25,000	10	10	20	40
	D	50,000	10	10	21	41

Statistical procedures employed: The mean and standard deviations of various measured parameters were calculated for each dose group. The significant difference between the control and the compound-treated groups was tested by using Student's t-analysis variance test. Those means showing significant differences have been marked with asterisks ($P<0.05$; *, $P<0.01$; **). The chi-square test of significance ($P<0.05$) by Mantel-Hanszel was employed to compare the survival date exclusive of sacrificed specimens. Prevalence rates were cited as percentages of tumor groups and non-tumor groups in cases of post-mortem examination. The significance of differences between the two means of prevalence was tested by using Fisher's exact test for fourfold tables. The percentages of the frequencies of tumor in specific tissues were analyzed by using the following technique: The Cochran-Armitage test for linear trend in proportion with continuity correction (Armitage[6]).

Physical examinations and observations: Animals were observed daily for survival. Body weights were recorded weekly for the first 55 weeks, bi-weekly thereafter. Food consumption was monitored weekly. Unusual signs, including indications of systemic pharmacologic or toxicologic effects, were routinely recorded at pre-specified styles and whenever necessary. At the end of 6- and 12- month periods, some specimens were sacrificed, to collect essential experimental data.

Clinical laboratory procedures: Hematologic and clinical chemistry examinations were performed on blood specimens of all animals collected via axillary puncture by employing vacuum at the end of 24-week (6 months) and 48-week (12 months) periods.

Determinations of the specimens were made for erythrocytes (RBC), hemoglobin (Hb), leukocytes (WBC), and hematocrit (Ht). A clinical chemistry was made for aspartate transaminase (AST), alanine transaminase (ALT), serum inorganic phosphorus (IP), total protein (TP), Albumin (ALB), lactic dehydrogenase (LDH), alkali phosphatase (ALP), total bilirubin (TB), total cholesterol (T-Cho), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), blood urea nitrogen (BUN), uric acid (UA), creatinine (Cre), and calcium (Ca) on serum separated from the blood after clotting.

Postmortem examination procedures: Animals were fasted overnight and sacrificed by etherization. Entire organs from the bodies were removed subsequent to gross examination of the lungs, bronchus, heart, kidneys, liver, spleen, brain, stomach, colon, intestines, pancreas, adrenal glands, pituitary, thyroid, salivary glands, thymus, testes, prostate, bladder, ovaries, uterus, oviducts, femoral bones, mammary glands, skin, and subcutis. The organs of the liver, kidneys, spleen, heart, and brain were weighed anew. Representative blocks of the above tissues were fixed in a cold, neutrally-buffered solution of formalin, and were embedded in paraffin, sectioned, stained with hematoxylin-eosin, and examined microscopically.

RESULTS

1. Mice

1) Body weight, food consumption, and survival

Growth rates, food consumption and compound dosages of orally-administered SYLOID are shown in Tables 2 and 3. Group mesns of growth rates are shown as percentages of the initial body weight in Figs. 1 and 2. During the initial 61-week period, the control and treated groups grew at essentially the same rate. No significant variations in body weight were observed throughout this study between the control and treated groups of 1.25% and 2.5% dosages. However, at the end of the initial 10-week period ($p<0.01$), the 5% dosage group showed lower growth rate as compared to the control group. At 81 weeks, an increase in food consumption in the control groups was evident in the treated male groups of 2.5% and 5% dosages. It is of interest to note that increased food consumption in the treated group of 5% dosage was accompanied by decreased body weight. The mean cumulative intake of SYLOID at the end of 93 weeks in the 1.25%, 2.5%, and 5% dietary levels was 38.45, 79.78, and 160.23 g/mouse in males, and 37.02, 72.46. and 157.59 g/mouse in females, respectively. The influences of compound ingestion on survival rates are shown in Figs.3 and 4. The group mean survival rates of the 5% dosage group was the greatest for both sexes. No significant difference in survival rates for each group was observed.

Most of the mice remained in good health, appeared to be active, and showed normal behavior throughout the treatment.

2) Clinical laboratory finding

Group means of hematology parameters at 6, 12, and 21 months evaluated for both sexes are shown in Tables 4-1 and 4-2. The mean HCT and MCV at 12 months in females showed a somewhat lower level in comparison with the normal group. However, there was no evidence of dose-related alteration of hematologic profiles at the end of the 12- and 21-month treatments.

3) Gross and microscopic findings

Group mean organ weights of the liver, kidneys, spleen, heart, and brain for mice-treated groups are shown in Tables 5-1 and 5-2. Abnormal atrophy or hypertrophy of the organs, which deviated significantly from the limits established by the normal group, was sporadically found only at the end of 6 weeks in the 2.5% and 5% dosage groups. However, none of these changes were sex- or dose-related.

Histopathological findings on neoplasms at termination within 21 months are summarized in Table 6. Tumors attributed to the treatment of SYLOID were found in the hematopoietic organs, particularly malignant lymphoma/leuiemia, which occurred in 7/20 (35%) in the female groups of the 2.5% dosage group. The results of the Cochran-Armitage test for positive dose-related trends in the incidence of tumors were not significant.

Table 2. Group mean body weights, food intake, and intake of SYLOID for male mice in lifespan study

Group		Weeks after feeding						
		0	5	15	30	50	81	93
0 (Control)	Body weight (g)	23.9±1.2	30.5±2.0	42.7±3.5	48.0±3.4	50.4±3.4	46.7±4.9	44.2±6.3
	Food intake (g/day)		5.0±0.55	5.2±0.54	4.7±0.74	4.7±0.21	4.2±0.29	3.8±0.70
1.25	Body weight (g)	24.8±1.1**	31.9±2.3**	42.9±3.8	48.9±4.0	50.2±4.0	45.0±3.7	40.5±6.1
	Food intake (g/day)		4.4±0.19*	5.6±0.61	4.9±0.18	4.8±0.05	4.3±0.62	3.7±0.73
	Daily intake of SYLOID(g/kg/day)		1.88	1.63	1.23	1.20	1.22	1.24
	Cumulative dose of SYLOID (g/mouse)		1.90	6.84	13.42	21.90	34.35	38.45
2.5	Body weight (g)	24.1±1.5	31.3±2.6	40.8±7.2	46.5±4.7	47.7±5.2*	44.0±6.1	41.5±6.3
	Food intake (g/day)		4.4±0.36*	5.9±0.74*	4.7±0.28	4.9±0.34	4.9±0.24**	4.7±0.34
	Daily intake of SYLOID (g/kg/day)		3.51	3.61	2.58	2.52	2.67	2.89
	Cumulative dose of SYLOID (g/mouse)		3.63	13.73	26.42	42.88	69.62	79.78
5.0	Body weight (g)	24.3±1.1	30.7±1.8	39.2±2.8**	44.1±3.9**	47.4±4.0**	44.5±4.2	43.4±5.4
	Food intake (g/day)		4.6±0.21	5.3±0.42	4.8±0.23	4.9±0.18	5.2±0.26**	4.7±0.48
	Daily intake of SYLOID (g/kg/day)		7.49	7.14	5.44	5.27	6.07	5.53
	Cumulative dose of SYLOID (g/mouse)		7.64	26.77	52.68	86.59	139.15	160.23

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 3. Group mean body weights, food intake, and intake of SYLOID for female mice in lifespan study

Group		Weeks after feeding						
		0	5	15	30	50	81	93
0 (Control)	Body weight (g)	18.3±0.8	23.8±1.8	35.0±4.3	42.9±5.3	50.9±5.3	54.8±4.4	53.3±7.6
	Food intake (g/day)		4.3±0.59	7.4±1.80	4.0±0.40	4.4±0.64	4.3±0.83	3.4±0.49
1.25	Body weight (g)	18.5±1.0	23.0±1.7**	35.0±4.4	42.7±5.6	48.9±5.4	55.3±4.5	54.7±6.1
	Food intake (g/day)		3.6±0.26**	7.1±1.46	3.7±0.27	3.9±0.24	4.7±0.39	4.1±0.36
	Daily intake of SYLOID (g/kg/day)		2.17	2.57	1.17	1.02	1.08	1.91
	Cumulative dose of SYLOID (g/mouse)		1.49	6.87	12.64	19.71	32.26	37.02
2.5	Body weight (g)	18.9±0.8**	23.4±1.5	35.3±3.2	42.6±4.5	49.0±4.8	56.1±4.7	54.8±6.5
	Food intake (g/day)		3.6±0.28**	7.1±1.94	3.4±0.36*	4.1±0.16	4.3±0.14	4.1±0.15*
	Daily intake of SYLOID (g/kg/day)		3.85	4.90	1.86	2.04	1.96	1.82
	Cumulative dose of SYLOID (g/mouse)		2.89	13.73	25.40	39.07	63.34	72.46
5.0	Body weight(g)	18.8±0.9	23.3±1.5	33.8±3.8	39.5±5.8*	47.1±6.6*	55.7±4.3	55.7±5.8
	Food intake (g/day)		3.9±0.38	8.1±0.94	3.8±0.38	4.3±0.41	4.8±0.05	4.4±0.41*
	Daily intake of SYLOID (g/kg/day)		8.58	13.31	4.81	4.67	4.31	3.95
	Cumulative dose of SYLOID (g/mouse)		6.10	29.97	56.75	86.56	138.79	157.59

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

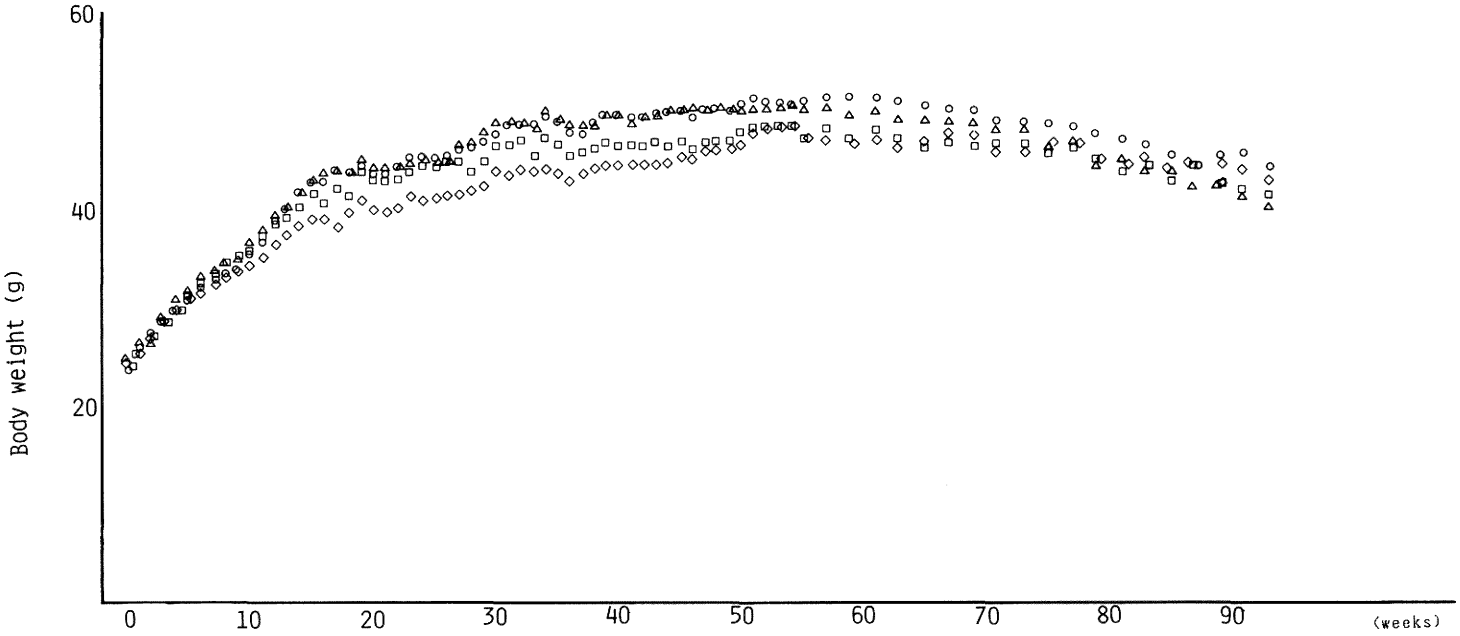

Fig. 1. Growth curves for male mice by dose (○; control, △; 1.25%, □; 2.5%, ◇; 5.0%)

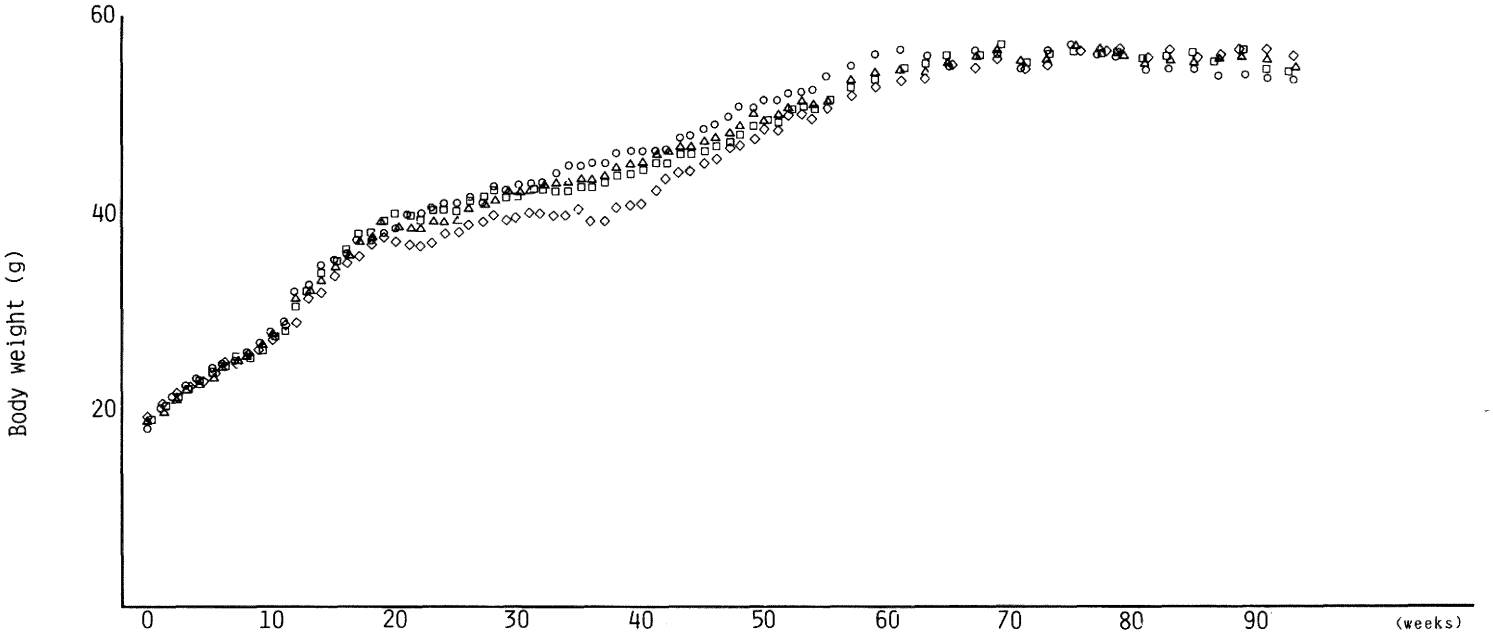

Fig. 2. Growth curves for female mice by dose (○; control, △; 1.25%, □;2.5%, ◇; 5.0%)

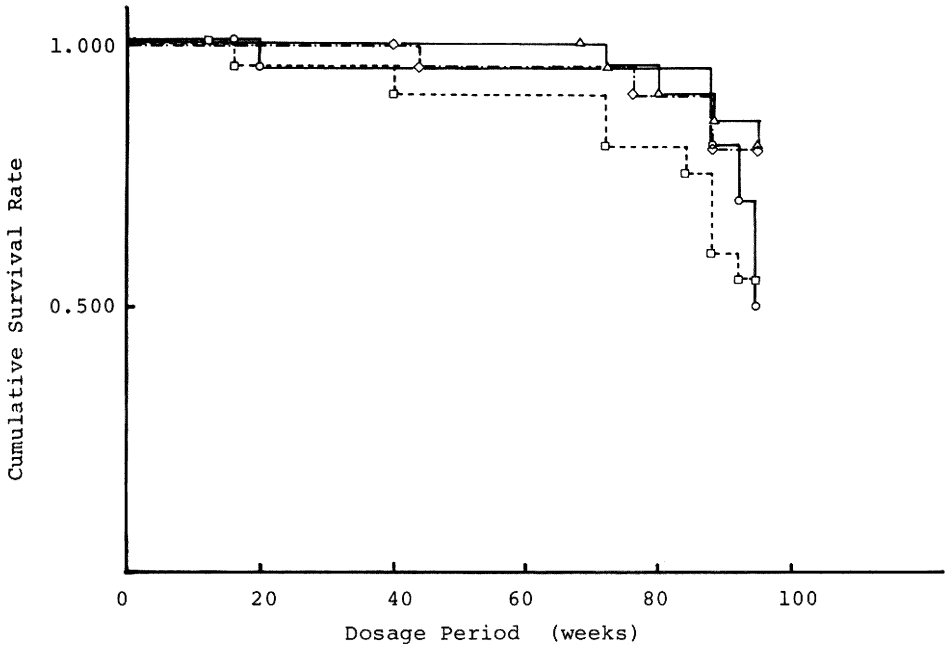

Fig. 3. Survival curves for male mice by dose (—○—; control, —△—; 1.25%, ···□···; 2.5%, --◇--; 5.0%)

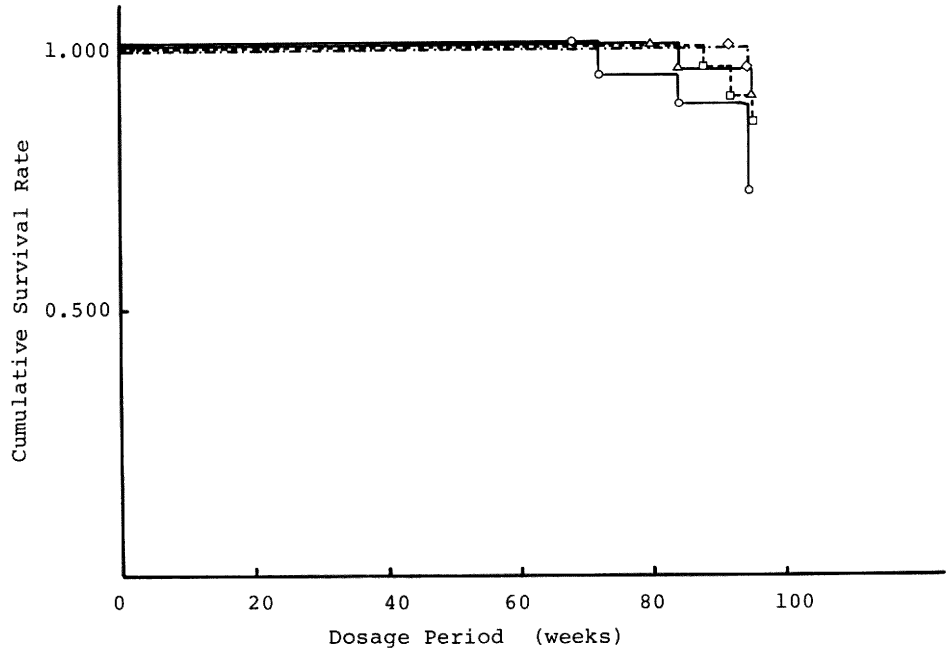

Fig. 4. Survival curves for female mice by dose (—○—; control, —△—; 1.25%, ···□···; 2.5%, --◇--; 5.0%)

Table 4-1. Blood chemistry observations of mice by sex (Male)

Dosage period (M)	Group (%)	No. of animals	R B C						WBC	Platelet
			Count (10^4/dl)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (%)	($\times 10^3$/dl)	($\times 10^3$/dl)
	0	6	1005.0± 50.3	15.1±0.59	49.7± 1.87	50.7±0.82	15.4±0.31	30.4±0.15	5.40±2.50	1354.0± 81.5
6	1.25	8	989.0± 42.3	15.2±0.62	50.7± 2.16	51.9±1.13	15.1±0.29	30.0±0.28	5.30±1.67	1191.0±118.0
	2.5	1								
	5.0	4	960.0±143.0	14.8±1.89	48.8± 7.62	52.0±1.63	15.9±0.19	30.5±1.09	4.00±2.64	686.0± 35.7
	0	7	1011.5± 58.2	13.4±3.15	58.6± 4.13	59.6±3.21	15.1±0.72	25.3±0.30	4.20±1.34	1519.0±164.5
12	1.25	7	1035.0± 52.3	14.8±0.44	59.1± 1.71	59.7±1.25	14.9±0.29	25.0±0.21	5.60±2.44	1406.0±187.5
	2.5	5	1051.0± 41.7	14.7±0.90	58.1± 3.82	58.2±3.56	14.8±0.89	25.4±0.37	3.60±1.08	1802.0±115.4**
	5.0	7	1052.0± 91.2	15.2±1.06	60.4± 4.31	61.3±3.30	15.4±0.83	25.1±0.47	4.30±0.92	1304.0±289.5
	0	9	896.3± 44.4	13.2±0.58	49.1± 1.78	54.9±1.64	14.7±0.27	26.8±0.45	7.10±2.93	1430.0±156.0
21	1.25	12	1126.0±303.2*	14.9±2.49*	54.8± 7.42*	58.3±4.50*	15.5±1.14*	26.8±0.63	5.30±2.37	1410.0±321.9
	2.5	10	854.9± 49.1	12.7±0.90	50.5± 7.62	56.9±5.07	15.0±0.96	27.0±0.70	7.20±1.80	1559.0±152.9
	5.0	13	762.4±195.3*	12.3±2.94	44.7±11.10	55.5±2.11	16.1±1.85*	27.3±0.61	6.76±2.77	1344.0±234.8

Mean±SD was calculated by the method of rejection limit from Smirnov

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 4-2. Blood chemistry observations of mice by sex (Female)

Dosage period (M)	Group (%)	No. of animals	R B C						WBC	Platelet
			Count (10^4/dl)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (%)	($\times10^3$/dl)	($\times10^3$/dl)
	0	1								
6	1.25	7	1028.0± 43.6	15.9±0.53	53.2±1.69	53.4±1.27	16.0±0.43	29.9±0.43	4.30±1.10	978.0± 77.6
	2.5	0								
	5.0	4	1004.0± 28.0	15.8±0	51.9±0.75	52.2±0.50	15.9±0.10	30.5±0.44		1075.0±299.5
	0	9	936.2± 24.4	14.3±0.41	55.5±1.67	59.2±1.20	15.3±0.20	25.8±0.19	3.40±2.30	1213.0±130.9
12	1.25	7	964.3± 32.7	14.5±0.40	56.9±1.59	59.0±1.29	15.2±0.36	25.7±0.17	4.30±1.57	1170.0±135.1
	2.5	9	952.2± 53.4	14.6±0.85	56.6±3.23	59.4±1.01	15.3±0.27	25.8±0.37	2.20±0.84	1070.0±148.4
	5.0	8	913.5± 73.5	14.5±0.82	45.9±8.12*	49.9±5.03**	15.9±0.86	32.2±4.84**	2.30±0.69	941.1±164.2
	0	12	929.5± 97.4	14.2±0.82	52.7±4.89	59.3±2.2	15.6±0.56	26.4±0.37	5.00±2.44	902.1±262.8
21	1.25	17	884.0± 91.2	14.1±0.91	54.5±2.33	60.1±1.76	15.7±0.37	26.2±0.22	2.80±0.81*	1035.0±159.3
	2.5	17	869.8±100.0	14.1±0.52	54.3±1.29	59.8±1.17	15.6±0.12	26.2±0.32	3.70±1.89	973.4±268.7
	5.0	14	823.6±181.4	14.1±0.88	54.3±1.80	59.7±1.25	15.7±0.42	26.3±0.33	3.50±1.22	897.2±130.6

Mean±SD was calculated by the method of rejection limit from Smirnov

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Teble 5-1. Group mean organ weights of liver, kidney, spleen, heart, and brain for mice (Male)

Dosage Period (M)	Group (%)	No.of animals	Organ Weight (g)				
			Liver	Kidney	Spleen	Heart	Brain
6	0	10	1.54±0.20	0.31±0.04	0.10±0.02	0.19±0.02	0.47±0.02
	1.25	10	1.51±0.13	0.32±0.03	0.10±0.01	0.19±0.02	0.48±0.02
	2.5	10	1.74±0.23	0.36±0.03**	0.11±0.01	0.21±0.02*	0.45±0.03
	5.0	10	1.53±0.19	0.34±0.03	0.08±0.009*	0.19±0.02	0.45±0.03
12	0	10	1.65±0.08	0.35±0.02	0.11±0.02	0.22±0.02	0.44±0.03
	1.25	10	1.81±0.14**	0.35±0.03	0.12±0.02	0.22±0.02	0.48±0.04*
	2.5	10	1.75±0.12	0.32±0.02**	0.10±0.01	0.20±0.02**	0.47±0.02*
	5.0	10	1.66±0.16	0.35±0.02	0.10±0.01	0.21±0.02	0.47±0.04
21	0	10	1.82±0.33	0.32±0.05	0.12±0.03	0.23±0.02	0.49±0.03
	1.25	16	2.07±0.90	0.33±0.06	0.12±0.05	0.21±0.04	0.46±0.03*
	2.5	11	1.86±0.25	0.34±0.07	0.13±0.04	0.21±0.02*	0.45±0.06
	5.0	16	1.81±0.16	0.36±0.06	0.13±0.04	0.22±0.03	0.46±0.03*

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Teble 5-2. Group mean Organ weights of liver, kidney, spleen, heart, and brain for mice (Female)

Dosage Period (M)	Group (%)	No.of animals	Organ Weight (g)				
			Liver	Kidney	Spleen	Heart	Brain
6	0	10	1.39±0.19	0.27±0.05	0.11±0.03	0.16±0.02	0.50±0.03
	1.25	10	1.28±0.17	0.23±0.04	0.10±0.02	0.15±0.01	0.49±0.02
	2.5	10	1.31±0.12	0.23±0.02*	0.11±0.02	0.13±0.01**	0.49±0.04
	5.0	10	1.17±0.08**	0.21±0.03**	0.09±0.009	0.13±0.01**	0.46±0.02**
12	0	10	0.51±0.22	1.27±0.05	0.11±0.02	0.17±0.02	0.48±0.04
	1.25	10	1.49±0.24	0.23±0.03*	0.11±0.02	0.16±0.03	0.49±0.01
	2.5	10	1.43±0.19	0.21±0.01**	0.09±0.02*	0.15±0.01*	0.52±0.02*
	5.0	10	1.37±0.14	0.24±0.02	0.10±0.01	0.14±0.01*	0.48±0.01
21	0	13	1.66±0.29	0.26±0.01	0.22±0.06	0.16±0.03	0.49±0.02
	1.25	18	1.81±0.36	0.26±0.05	0.18±0.04	0.18±0.04	0.49±0.03
	2.5	17	1.91±0.38	0.34±0.05**	0.24±0.08	0.17±0.02	0.46±0.03**
	5.0	19	1.76±0.29	0.28±0.03*	0.19±0.08	0.17±0.02	0.48±0.03

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 6. Incidence of tumors in mice fed SYLOID for two years

Site and tumor type	Period*1 (month)	Control	1.25%	2.5%	5.0%	Trend analysis Cochran-Armitage
Liver	6	0/10	0/10	0/10	0/10	—
hyperplastic nodule	12	3/10(30.0)	1/10(10.0)	2/10(20.0)	0/10	NS
	21	2/16(12.5)	8/17(47,1)	5/14(35.7)	4/16(25.0)	NS
Lung	6	0/10	0/10	0/10	0/10	—
adenoma	12	1/10	1/10	1/10	0/10	NS
adenocarcinoma	21	1/16(6.25)	2/17(11.8)	3/14(21.4)	3/16(18.8)	NS
Hematopoietic	6	0/10	0/10	0/10	0/10	—
leukemia	12	0/10	0/10	0/10	0/10	—
malignant lymphoma	21	3/16(18.8)	1/17(5.9)	0/14	1/16(6.25)	NS
Liver	6	0/10	0/10	0/10	0/10	—
hyperplastic nodule	12	0/10	0/10	0/10	0/10	—
	21	3/16(18.8)	3/19(15.8)	1/20(5.0)	3/20(15.0)	NS
Lung	6	0/10	0/10	0/10	0/10	—
adenoma	12	0/10	0/10	2/10(20.0)	0/10	NS
adenocarcinoma	21	0/16	1/19(5.3)	0/20	1/20(5.0)	NS
Hematopoietic	6	0/10	0/10	0/10	0/10	—
leukemia	12	0/10	0/10	0/10	0/10	—
malignant lymphoma	21	2/16(12.5)	4/19(21.1)	7/20(35.0)	2/20(10.0)	NS

NS; not significantly different —; not measured

In the lungs, the frequency of adenoma/adenocarcinoma was 1/16 (6.25%) for the control, 2/17 (11.8%) for the 1.25%, 3/14 (21.4%) for the 2.5%, and 3/16 (18.8%) for the 5% dosage groups of males. The incidence of the lung adenomas in females was greater than that of males. However, none of these findings were sex- or dose-related.

In the liver, the correlation of hyperplastic nodules/hepato cellular carcinoma/ hemangioma/fibrosarcoma in the treated groups, as compared with the control group, was relatively low.

Non-neoplastic lesions were observed in the subcutis, lungs, kidneys, and liver in the treated groups. But these were considered to be of no toxicological significance.

2. Rats

1) Body weight, food consumption, and survival

Group mean growth rates, food consumptions, and compound intake by sex are shown in Tables 7, 8 and Figs.5. and 6. No consistent compound- or dose-related changes in these parameters were evident.

The mean cumulative intake of SYLOID at the end of 103 weeks for the 1.25. 2.5. and 5% dietary levels was 143.46, 179.55, and 581.18 g/rat in males, and 107.25, 205.02 and 435. 33 g/rat in females, respectively.

In male groups some deaths occurred over a period of 48 weeks. The mean survival rates found in treated groups, as is evident from Fig. 7. was greatest in the 5%, followed by the control and 1.25 or 2.5% dosage groups. However, no significant variations in survival rates were observed between the control and treated male groups. While the female survival rates of 5%, 2.5%, and 1.25% groups (shown in Fig. 8) were 0.875, 0.80, and 0.65 respectively, these were not statistically and significantly different from the values observed in the control group. None of these survival rates were dose-related.

No physical or behavioral signs of pharmacologic effects were observed during the treatment.

2) Clinical laboratory findings

Group means of hematology parameters at the end of 6-, 12-, and 24-month periods for both sexes are shown in Tables 9-1 and 9-2. Occasional erratic variations in hematologic profiles were observed in the treated groups: high WBC at 24 months in male groups of 1.25% dosage, and low RBC, HGB, and HCT at 24 months in the female 2.5% dosage group. The very high value for WBC in male rats of the 1.25% group looks as if it has one or two values, perhaps because of technical errors. However, no significance can be attached to the difference.

Group means of serum biochemistry parameters at the end of 6-, 12- and 24-month periods for both sexes evaluated are omitted from the table. No biologically meaningful changes in TA, ALB, AST, ALT, ALP, T-BL, and LDH were observed, although transient differences reaching statistical significance were frequently present. No noteworthy changes related to compound ingestion were observed in any parameters of renal analyses, such as BUN, CRE, and UA.

Table 7. Group mean body wights, food intake, and intake of SYLOID for male rats in lifespan study

Group		Weeks after feeding						
		0	5	15	30	50	81	103
0 (Control)	Body weight (g)	134.6± 7.8	273.5±11.8	376.3±16.4	428.9±20.4	470.0±27.4	455.7±37.0	420.0±55.6
	Food intake (g/day)		15.9± 0.48	15.1± 0.38	15.2± 0.78	15.4± 0.65	14.7± 0.41	15.6± 2.93
1.25	Body weight (g)	134.2± 9.5	280.5±13.0*	396.6±18.9**	458.9±22.4**	498.5±24.6**	467.6±36.9	453.2±65.6
	Food intake (g/day)		16.4± 0.34*	16.1± 0.52**	16.4± 0.70*	16.1± 0.19*	15.2± 0.82	16.3± 1.45
	Daily intake of SYLOID (g/kg/day)		0.71	0.50	0.46	0.40	0.41	0.44
	Cumulative dose of SYLOID (g/rat)		6.71	21.19	42.96	72.58	115.64	143.46
2.5	Body weight (g)	134.3± 8.5	274.0±11.7	386.3±16.4**	438.8±19.2	477.9±19.6	464.4±24.8	429.2±67.1
	Food intake (g/day)		16.0± 0.39	15.9± 0.48**	15.5± 0.88	16.2± 0.78	15.1± 1.06	14.5± 3.39
	Daily intake of SYLOID (g/kg/day)		1.46	1.04	0.89	0.86	0.83	0.85
	Cumulative dose of SYLOID (g/day)		12.95	41.05	81.44	137.68	225.01	179.55
5.0	Body weight (g)	133.4± 6.9	272.9±11.4	377.2±16.0	427.7±17.2	458.7±27.3	464.8±31.6	423.6±70.1
	Food intake (g/day)		16.5± 0.56*	15.9± 0.47**	15.6± 0.43	16.2± 0.33*	16.2± 0.75*	15.4± 2.93
	Daily intake of SYLOID (g/kg/day)		3.0	2.12	1.85	1.76	1.77	1.83
	Cumulative dose of SYLOID (g/rat)		27.24	84.01	166.29	280.37	463.22	581.18

*; Significantly different ($P<0.05$) **; Significantly difierent ($P<0.01$)

Table 8. Group mean body wights, food intake, and intake of SYLOID for female rats in lifespan study

Group		Weeks after feeding						
		0	5	15	30	50	81	103
0 (Control)	Body weight (g)	112.0± 5.9	176.9± 7.3	225.5±11.2	258.8±12.9	327.0±19.8	371.4±30.0	391.0±21.8
	Food intake (g/day)		10.4± 0.28	10.0± 0.35	10.2± 0.85	11.3± 0.36	12.1± 0.62	15.4± 4.08
1.25	Body weight (g)	110.9± 6.0	174.0± 8.8	222.9±12.9	257.7±17.4	323.3±33.2	383.4±34.0	402.2±70.4
	Food intake (g/day)		10.3± 0.27	9.8± 0.38	10.4± 0.53	11.9± 0.37*	12.4± 0.32	14.9± 2.84
	Daily intake of SYLOID (g/kg/day)		0.75	0.54	0.50	0.46	0.40	0.48
	Cumulative dose of SYLOID (g/rat)		4.70	13.67	27.56	48.40	82.85	107.25
2.5	Body weight (g)	110.0± 6.0	173.0± 8.4*	221.0±10.7	248.6±15.0**	306.1±24.1**	357.2±27.7	360.9±47.4*
	Food intake (g/day)		10.2± 0.45	9.8± 0.27	9.8± 0.42	11.8± 0.76	11.7± 1.43	13.0± 1.70
	Daily intake of SYLOID (g/kg/day)		1.45	1.13	1.00	0.98	0.83	0.88
	Cumulative dose of SYLOID (g/rat)		9.38	27.26	53.60	93.31	159.34	205.02
5.0	Body weight (g)	108.5± 6.0**	174.2± 8.8	223.2±10.8	252.9±10.4	309.7±17.7**	363.6±25.7	358.5±56.4*
	Food intake (g/day)		11.1± 0.42**	10.4± 0.37*	10.2± 0.46	11.9± 0.84*	13.2± 0.83	12.7± 2.67
	Daily intake of SYLOID (g/kg/day)		3.21	2.33	2.02	1.94	1.81	1.78
	Cumulative dose of SYLOID (g/rat)		20.28	58.45	114.18	197.53	335.19	435.33

*; Significantly different ($P<0.05$) **; Significantly diffierent ($P<0.01$)

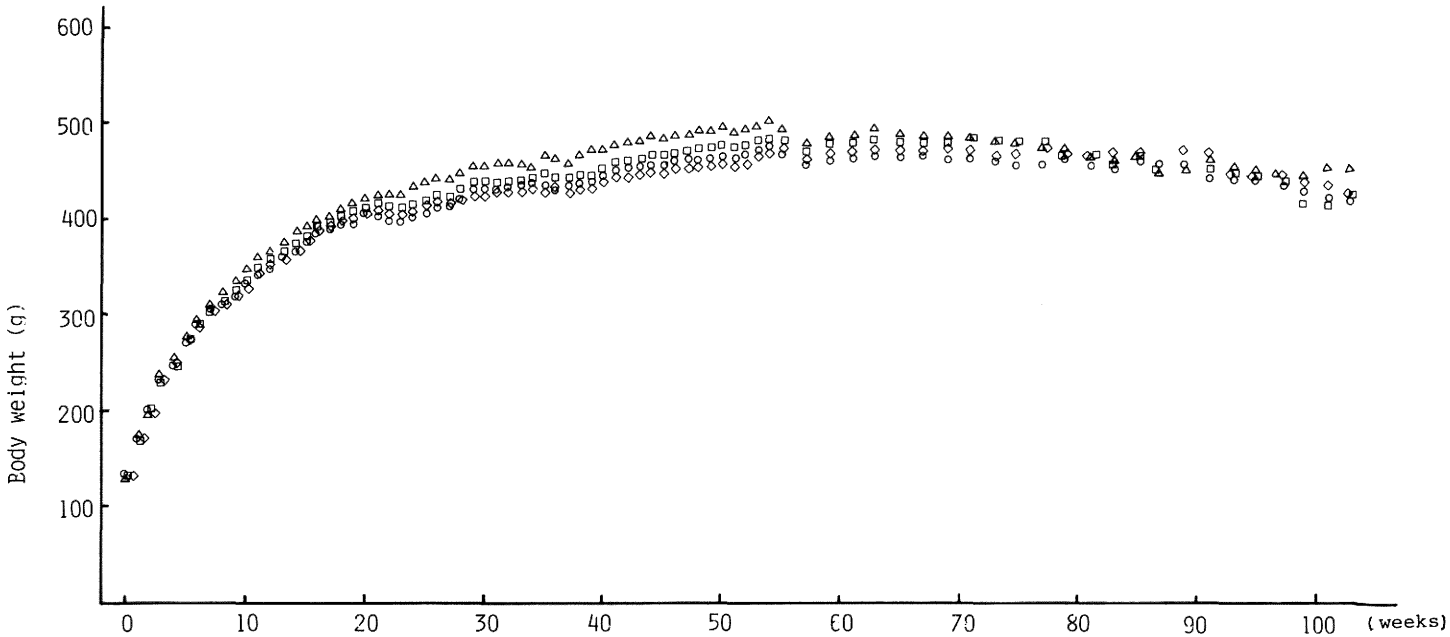

Fig. 5. Growth curves for male rats fed various diets (○; control, △; 1.25%, □; 2.5%, ◇; 5.0%)

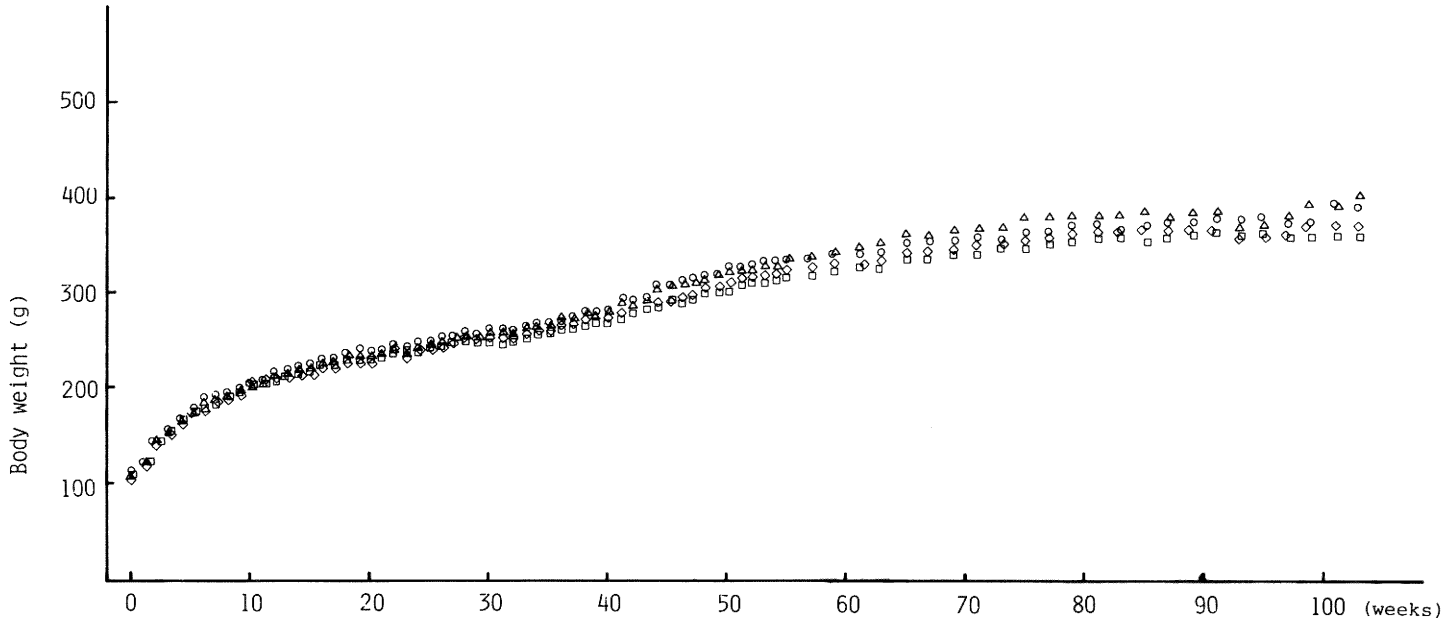

Fig. 6. Growth curves for female rats fed various diets (○; control, △; 1.25%, □; 2.5%, ◇; 5.0%)

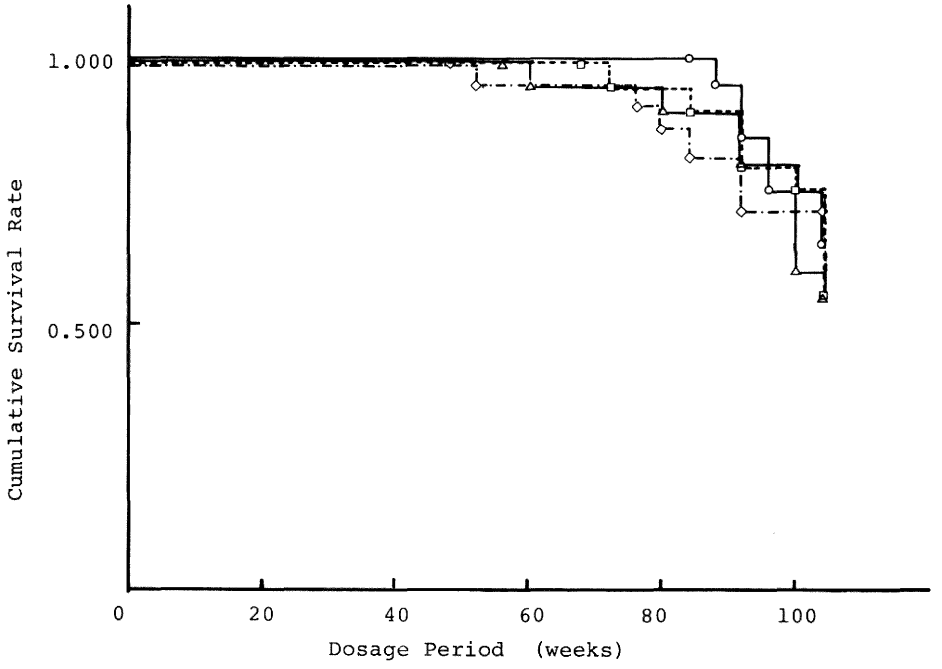

Fig. 7. Survival curves for male rats by dose (—○—; control, —△—; 1.25%, ···□···; 2.5%, ---◇---; 5.0%)

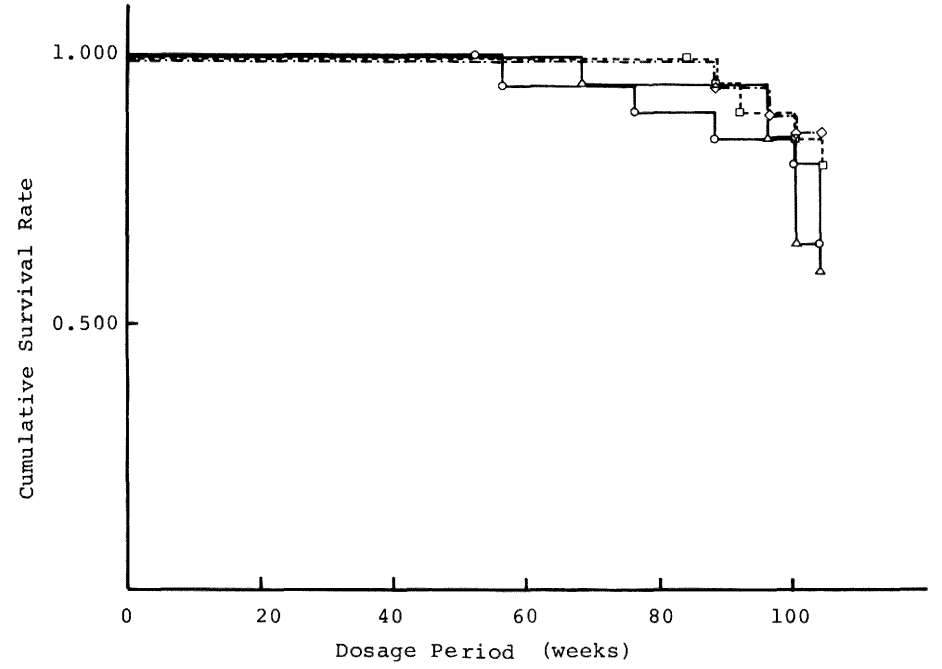

Fig. 8. Survival curves for female rats by dose (—○—; control, —△—; 1.25%, ···□···; 2.5%, ---◇---; 5.0%)

Table 9-1. Hematological observations of rats by sex (Male)

Dosage period (M)	Group (%)	No. of animals	R B C					WBC	Platelet
			Count (10^4/dl)	HGB (g/dl)	HCT (%)	MCH (pg)	MCHC (%)	($\times10^3$/dl)	($\times10^3$/dl)
	0	8	974.3± 59.0	16.5±0.92	51.4±3.07	17.1±0.08	32.3±0.20	5.30± 2.48	642.9± 59.4
6	1.25	10	1003.0± 18.1	16.7±0.35	52.4±1.89	16.7±0.35**	31.8±0.25**	6.20± 1.16	731.6± 44.1**
	2.5	8	965.3± 29.4	16.7±0.33	50.9±1.29	17.2±0.32	32.6±0.64	5.70± 1.87	688.3± 44.9
	5.0	9	993.0± 24.8	16.6±0.79	51.9±1.97	16.9±0.20*	31.9±0.65	5.16± 1.19	686.0± 35.7
	0	10	1011.0± 41.5	16.1±0.72	64.0±2.86	16.2±0.73	25.1±0.16	3.9± 1.30	627.4± 54.8
12	1.25	10	983.6± 9.4	16.2±0.37	64.6±1.03	16.4±0.33	25.0±0.31	4.7± 0.85	631.0± 28.0
	2.5	10	979.3± 16.0	16.2±0.21	64.7±0.91	16.5±0.24	25.1±0.17	5.1± 0.94*	671.4± 25.8
	5.0	9	973.1± 41.3	15.9±0.61	63.1±2.39	16.3±0.21	25.2±0.29	4.5± 0.38	662.9± 26.4
	0	12	810.4±222.3	11.8±3.51	45.6±14.5	14.6±3.65	25.7±0.56	8.5± 6.26	991.2±374.9
24	1.25	9	740.9±142.0	12.2±3.05	48.5±12.2	16.9±0.63	25.2±0.35**	23.3±22.70	716.0±177.4
	2.5	10	775.5±286.2	12.7±5.49	48.6±20.9	16.3±4.28	26.2±1.89	4.5± 0.91	689.9± 71.4*
	5.0	13	723.1±226.6	11.8±4.21	45.8±17.3	15.6±3.25	26.2±2.38	7.2± 4.62	695.1±239.7*

Mean±SD was calculated by the method of rejection limit form Smirnov

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 9-2. Hematological observations of rats by sex (Female)

Dosage period (M)	Group (%)	No. of animals	R B C					WBC	Platelet
			Count (10^4/dl)	HGB (g/dl)	HCT (%)	MCH (pg)	MCHC (%)	($\times10^3$/dl)	($\times10^3$/dl)
	0	6	894.8± 36.0	16.6±0.73	51.1± 2.24	18.6±0.25	32.5±0.61	3.38±0.83	696.8± 23.7
6	1.25	10	889.4± 24.4	16.4±0.55	52.0± 0.91	18.5±0.28	31.8±0.87	5.21±1.32**	683.9± 64.9
	2.5	9	883.0± 40.4	16.4±0.78	51.1± 2.38	18.6±0.20	32.1±0.80	4.01±1.68	700.0± 41.5
	5.0	9	883.6± 34.4	16.3±0.63	51.2± 2.44	18.4±0.42	31.8±0.85	4.58±1.61	648.3±194.3
	0	10	883.7± 14.6	15.7±0.25	62.5± 0.99	17.7±0.23	25.1±0.29	3.3 ±0.46	574.9± 30.1
12	1.25	9	876.3± 14.0	15.5±0.25	62.0± 1.04	17.7±0.21	25.0±0.23	3.1 ±0.55	598.1± 56.7
	2.5	9	880.0± 35.4	15.6±0.72	62.2± 2.41	17.7±0.26	25.1±0.35	3.4 ±0.29	601.0± 55.4
	5.0	9	890.0± 11.8	15.9±0.22	63.0± 0.73	17.8±0.44	25.2±0.14	3.0 ±0.60	606.9± 27.7*
	0	13	790.0± 33.8	14.4±0.56	55.7± 2.35	18.3±0.46	25.9±0.35	6.0 ±2.59	662.0±144.6
24	1.25	9	813.4± 39.2	14.6±0.68	57.2± 2.46	17.9±0.66	25.6±2.70	5.0 ±1.28	668.4±159.9
	2.5	14	668.0±155.3*	12.1±2.85*	46.5±11.50*	18.4±3.70	26.0±1.56	6.0 ±2.78	732.1±324.2
	5.0	16	785.9± 56.9	14.3±0.86	56.2± 2.52	18.2±0.54	25.8±0.42	4.8 ±1.96	741.9±127.7

Mean±SD was calculated by the method of rejection limit from Smirnov

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

3) Gross and microscopic findings

Chronic toxicity would require evidence of non-tumor pathology. Group mean organ weight values in postmortem observations at the end of 6-, 12- and 24-month periods for both sexes are shown in Table 10-1 and 10-2. Statistically significant lower liver weights were noted from 12 months to 24 months in the 2.5% and 5% female dosage groups. No noted atrophy or hypertrophy of the organs in each group was sex-or dose-related.

Histopathological examination of preliminary carcinogenicity of main neoplastic lesions is summarized in Tables 11 and 12. The incidence of tumors was the greatest in the genital organs, next in the skin. The other organs showed relatively low incidence. The histopathological findings of main tumors were as follows:

a. Adenomas/adenocarcinomas of mammary gland were found 7/19 (36.8%) in the control male group, 8/18 (44.4%) in the 1.25% group, 1/18 (5.6%) in the 2.5% group, and 1/17 (5.9%) in dosage male groups. The results of the Cochran-Armitage test for positive dose-related trends in the incidence of tumors of each group were not significant.

b. Seminomas of testes occurred highly in each treated male that died during the period of 12 to 24 months. However, no significance can be attached to these findings.

c. Incidences of adenomas in the prepuce/clitoris were greater than those in the reports by Sakai and Nagao[7] and Maekawa et al.[8]. The incidence of clitoral adenomas in male rats varied according to dosage; those in the control group showed the highest value of 10/17 (58.8%), followed by 8/17 (47.1%) for the 1.25% dosage group, 7/20 (35%) for the 2.5% dosage group, and 5/21 (23.8%) for the 5% dosage group. The result of the Cochran-Armitage test for negative dose-related trends in the females was significant ($P<0.05$). However, the incidences showed no significant differences between the control and treated groups.

DISCUSSION

Silicon dioxide and various silicates are present in all bodies of water, plants, and animals. Silicon has been demonstrated to be an essential trace element for vertebrae (Carlisle[4]).

Earlier work showing that silica dust is harmful to man is based largely upon epidemiological investigations of miner's phthisis and other allied forms of occupational tuberculosis (King and Belt[5]). This study was conducted to evaluate and characterize the effects of silica-SYLOID as food ingredients on $B_6C_3F_1$ mice and Fisher rats. Data collected from a 93-or 103-week chronic oral toxicity experiment, including carcinogenicity of SYLOID, were obtained by administering the test compound to 3-or 4-week-old animals on a continuous diet. The test compound was administered at daily intake levels of 0 (control), 1.25, 2.5, and 5.0% dosages. Control animals received only the test compound free-basal diet. Physical examinations, clinical laboratory examina-

Table 10-1. Group mean organ weights of liver, kidney, spleen, heart, and brain for rats (Male)

Dosage Period (M)	Group (%)	No.of animals	Organ Weight (g)				
			Liver	Kidney	Spleen	Heart	Brain
6	0	10	9.08±0.39	1.09±0.03	0.69±0.03	1.02±0.13	1.96±0.05
	1.25	10	10.10±0.61**	1.10±0.05	0.77±0.05**	1.05±0.07	2.00±0.04
	2.5	10	9.41±0.39	1.16±0.14	0.75±0.06*	1.01±0.05	1.85±0.08**
	5.0	10	9.43±0.97	1.12±0.07	0.70±0.03	1.06±0.09	2.02±0.07*
12	0	10	9.76±0.32	1.19±0.08	0.81±0.05	1.17±0.06	1.93±0.10
	1.25	10	10.99±0.78**	1.23±0.08	0.90±0.08**	1.22±0.08	2.09±0.04**
	2.5	10	10.26±0.62	1.17±0.08	0.85±0.04	1.12±0.05	1.92±0.07
	5.0	10	10.24±0.51**	1.26±0.06**	0.81±0.05	1.10±0.05**	2.13±0.08**
21	0	13	12.42±2.09	1.32±0.16	1.19±0.36	1.55±0.24	2.06±0.11
	1.25	11	13.20±1.20	1.50±0.21*	2.64±1.66*	1.52±0.21	2.08±0.06
	2.5	11	12.10±1.10	1.35±0.11	1.24±0.32	1.45±0.29	2.03±0.05
	5.0	15	12.45±2.60	1.54±0.59	1.69±1.09	1.52±0.19	2.01±0.11

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Table 10-2. Group mean organ weights of liver, kidney, spleen, heart, and brain for rats (Female)

Dosage Period (M)	Group (%)	No.of animals	Organ Weight (g)				
			Liver	Kidney	Spleen	Heart	Brain
6	0	10	5.42±0.57	0.76±0.09	0.52±0.03	0.74±0.08	1.86±0.07
	1.25	10	5.68±0.51	0.79±0.08	0.51±0.03	0.75±0.06	1.91±0.04
	2.5	10	5.44±0.33	0.70±0.05	0.49±0.03*	0.67±0.06*	1.08±0.07
	5.0	10	5.30±0.37	0.70±0.07	0.47±0.03**	0.64±0.05**	1.80±0.05**
12	0	10	7.52±0.24	0.84±0.05	0.63±0.04	0.77±0.05	1.86±0.07
	1.25	10	7.18±0.52	0.77±0.07*	0.62±0.04	0.77±0.07	1.89±0.05
	2.5	10	6.96±0.43**	0.79±0.06	0.57±0.04**	0.77±0.04	1.80±0.08
	5.0	10	7.01±0.29**	0.80±0.06	0.58±0.04	0.74±0.06	1.92±0.10
21	0	13	11.23±1.34	1.03±0.12	0.87±0.26	0.91±0.10	1.86±0.07
	1.25	12	11.13±2.33	1.09±0.09	0.84±0.16	0.95±0.07	1.91±0.09
	2.5	16	9.69±1.32**	1.01±0.08	2.12±2.70	0.92±0.09	1.84±0.08
	5.0	18	9.57±1.51**	1.06±0.13	0.80±0.19	0.88±0.09	1.81±0.12

*; Significantly different ($P<0.05$) **; Significantly different ($P<0.01$)

Teble 11. Incidence of tumors in male rats fed SYLOID for two years

Site and tumor type	Period*1 (month)	Control	1.25%	2.5%	5.0%	Trend analysis Cochran-Armitage
Liver	6	0/10	0/10	0/10	0/10	—
hyperplastic nodule	12	0/10	0/10	0/10	0/10	—
	24	0/19	1/18(5.6)	0/18	2/17(11.8)	NS
Lung	6	0/10	0/10	0/10	0/10	—
adenoma	12	1/10	0/10	1/10	0/10	NS
adenocarcinoma	24	1/19	0/18	0/18	0/17	NS
Hematopoietic	6	0/10	0/10	0/10	0/10	—
leukemia	12	0/10	0/10	0/10	0/10	—
	24	0/19	1/18(5.6)	1/18(5.6)	1/17(5.9)	NS
Adrenal	6	0/10	0/10	0/10	0/10	—
Pheochromocytoma	12	0/10	0/10	0/10	0/10	—
	24	0/19	1/18	2/18(11.1)	2/17(11.8)	NS
Teste	6	0/10	0/10	0/10	0/10	—
seminoma	12	0/10	1/10	0/10	0/10	NS
	24	15/19(78.9)	14/18(77.8)	14/18(77.8)	14/17(82.4)	NS
Mammary gland	6	0/10	0/10	0/10	0/10	—
adenoma	12	0/10	0/10	0/10	0/10	—
adenocarcinoma	24	7/19(36.8)	8/18(44.4)	1/18(5.6)@	1/17(5.9)@	$P<0.01$
Prepuce	6	0/10	0/10	0/10	0/10	—
fibroma	12	0/10	0/10	0/10	3/10	NS
	24	5/19(26.3)	3/18(16.7)	4/18(22.2)	4/17(23.5)	NS

—: Not tested, NS; Not significantly different ($P<0.05$) @; Significantly different ($P<0.01$)

Teble 12. Incidence of tumors in female rats fed SYLOID for two years

Site and tumor type	Period*1 (month)	Control	1.25%	2.5%	5.0%	Trend analysis Cochran-Armitage
Liver	6	0/10	0/10	0/10	0/10	—
hyperplastic nodule	12	0/10	0/10	0/10	0/10	—
	24	0/17	0/17	0/20	1/21	NS
Lung	6	0/10	0/10	0/10	0/10	—
adenoma	12	0/10	0/10	0/10	0/10	—
adenocarinoma	24	0/17	0/17	2/20(10.0)	0/21	NS
Hematopoietic	6	0/10	0/10	0/10	0/10	—
leukemia	12	0/10	0/10	0/10	0/10	—
malignant lymphoma	24	1/17(5.9)	1/17(5.9)	3/20(15.0)	0/20(0.0)	NS
Mammary gland	6	0/10	0/10	0/10	0/10	—
adenoma	12	0/10	1/10	0/10	0/10	NS
adenocarcinoma	24	8/17(47.1)	9/17(52.9)	11/20(55.0)	10/21(47.6)	NS
Clitoris	6	0/10	0/10	0/10	0/10	—
adenoma	12	0/10	0/10	0/10	0/10	NS
	24	10/17(58.8)	8/17(47.1)	7/20(35.0)	5/21(23.8)	$P<0.05$

—; Not tested, NS; Not significantly different ($P<0.05$)

Numbers in panentheses are expressed as %

tions, gross postmortems, and microscopic examinations were performed on mice at the end of 30 weeks (ca 6 months), 50 weeks (ca 12 months), and 93 weeks; the examination period on rats was 103 weeks.

Data from this study suggest that mortality rates were unaffected by compound treatment; the survival rate of each group did not show dose-related differences. No compound-related variation in food consumption and body weight gain was observed. Physical examination findings were normal; no changes in behavioral activity or in general physical appearance were noted. Hematology and clinical chemistry findings revealed no biologically meaningful dose-related alterations, although transient sporadic significant differences in several of the parameters were observed. As mentioned above, most values remained within normal limits; no consistent dose-related pattern was evident. Gross postmortem and microscopic findings were normal. Evidence of incidental neoplasm was observed, as expected. However, no indication of dose-related alterations was present. Organ weight data were also normal.

As for amorphous silicon dioxide, (incl. silica aerogel, hydrated silica, silicic acid, and dehydrated silica gel) it is categorized as List A (1) (the Joint FAO/WHO Expert Committee on Food Additives,); consequently, the maximum level of the substance singly or in combination with calcium silicate, should not exceed 10g/kg of the salt substitute mixture.

The acute oral LD_{50} of SG67 (Market product of silicon) for male albino rats should be greater than 3160 mg/kg of body weight. Mice, rats, and guinea pigs were exposed for 6 hours to the dust of SG67 at a concentration of 0.0473 mg/1. No signs of toxicity were noted.

Although silicon is part of the normal human diet, the compounds consumed as food additives are only a minor portion of the total dietary intake of silicon. It is evident from the facts described above that the risks are extremely low with regard to normal intake.

In our judgment, these results suggest that the use of SYLOID as an anti-caking agent is safe for human consumption.

References

1) McClendon J. F., Gershon-Cohen J. & Brody H.: Absence of gastro-intestinal leisons in rats following ingestion of Silica. *Proc. Soc. Exp. Biol. Med.*, **95**: 330-331. 1958.
2) INBIFO: 6-monat-toxizitatsunter-suchung mit SYLOID; An ratten bei oraler applikation (Fotterungsversuch). *Report of INBIFO institut fur biologishe forshung (Koln)*, 1975.
3) Schwarz K. & Milne D. B.: Growth-promoting effects of silicon in rats. *Nature*, Lond., **239**: 333-334, 1972.
4) Carlisie E. M.: Silicon as essential element. Fed. Pro. Fed. *Am. Soc. Exp. Biol.* **33**; 1758-1766, 1974.
5) King E. J. & Belt T. H.: The physiological and pathological aspects of silica. *Physiol. Rev.*, **18**: 329-365, 1938.
6) Armitage P.: Statistical methods in medical research. *Black Well Sci. Publ. Lond. 4th ed. p. 462., 1987.*

7) *Sakai T. and Nagao. S.: Twenty-eight week toxicity study of Perlite powder in mice. J. Toxicol. Sci.,* 10: 83-93. 1985.

8) *Maekawa A., Takizawa H. & Hayashi Y.: Spontaneous tumors in mice, rats, and hamsters used as general mutagenicity test. *Toxicology Forum.* 4: 22-41, 1981.
(*written in Japanese)